

## DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

Attorney Docket No. 3339-239A

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

### METHOD FOR THE REGULATION OF PROTEIN BIOSYNTHESIS

the specification of which

☒ is attached hereto

OR

☐ was filed on \_\_\_\_\_ as United States Application No. or PCT International Application Number \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

92 06765	France	June 4, 1992	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Number	Country	MM/DD/YYYY Filed	Priority Claimed
			<input type="checkbox"/> Yes <input type="checkbox"/> No
Number	Country	MM/DD/YYYY Filed	Priority Claimed
			<input type="checkbox"/> Yes <input type="checkbox"/> No
Number	Country	MM/DD/YYYY Filed	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date (MM/DD/YYYY)
Application Number	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application (37 C.F.R. § 1.63(d)).

PCT/FR 92/00524	June 2, 1993	National Phase Entered
Application No.	Filing Date	Status Patented/Pending/Abandoned
08/347,353	December 1, 1994	Pending
Application No.	Filing Date	Status Patented/Pending/Abandoned
Application No.	Filing Date	Status Patented/Pending/Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney's Docket No. 3339-239A

Applicant, Patentee, or Identifier: Joel Sternheimer  
Application No. or Patent No.: To Be Assigned  
Filed or Issued: Concurrently Herewith  
Title: METHOD FOR THE REGULATION OF  
PROTEIN BIOSYNTHESIS

STATEMENT CLAIMING SMALL ENTITY STATUS  
(37 C.F.R. § 1.9(f) and 1.27(b))--INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. § 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office described in:

- ☒ the specification filed herewith with title as listed above.  
☐ the application identified above.  
☐ the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or nonprofit organization under 37 C.F.R. § 1.9(e).

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☒ No such person, concern, or organization exists.  
☐ Each such person, concern, or organization is listed below.

FULL NAME: \_\_\_\_\_

ADDRESS: \_\_\_\_\_

☐ Individual

☐ Small Business

☐ Nonprofit Organization

FULL NAME: \_\_\_\_\_

ADDRESS: \_\_\_\_\_

☐ Individual

☐ Small Business

☐ Nonprofit Organization

Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 C.F.R. § 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b))

Joel Sternheimer

NAME OF INVENTOR

Joel Sternheimer JSN

(Signature of Inventor)

Date

12/5/95

CLT01/4358044v1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Joël Sternheimer  
Appl. No.: To Be Assigned  
Filed: Concurrently Herewith  
For: METHOD FOR THE REGULATION OF  
PROTEIN BIOSYNTHESIS

May 25, 1999

Assistant Commissioner for Patents  
Washington, DC 20231

**RULE 132 DECLARATION  
OF JOËL STERNHEIMER**

Sir:

I, JOËL STERNHEIMER, do hereby declare and say as follows:

1. That I am a graduate of Paris, Lyons and Princeton Universities and received my degrees in the years 1964, 1966 and 1967 (Doctorate in 1966).  
My Curriculum Vitae and the list of publications are attached thereto (Annexes 1 and 2).
2. That it clearly emerges from the Invention as now defined that it does not lack utility: the method of the invention allows and controls the *in situ* regulation of the synthesis of selected protein. It was undertaken by Mr. Pedro Ferrandiz under my supervision to stimulate the growth of blue-green algae –prokaryotes, genus *Anabaena*- by epigenetic regulation. Their photosynthetic activity involves in particular pigmentary proteins (cyanins). Thus their biosynthesis is easily observed through color change and oxygen release.

We want to point out that this first attempt of stimulation in an aquatic medium is relatively simple to reproduce. We believe that the results obtained are particularly promising. One may add the fact that it points towards numerous applications.

- Materials and methods

- Dilution of 12 ml of *Anabaena variabilis* (stock provided by the Ecole Normale Supérieure de Paris) in 1 500 ml of mineral water.
  - Addition of 40 g of dry vegetable manure containing 8%, say 2.6 g, of nitrates as well as 40 g of river pebbles (as suggested by Vincent Bargoin this would provide the solution with trace elements).
  - Adaptation time to the cultures medium: four days.
  - Transfer of 750 ml of the solution in two vats subjected to natural enlightenment.
- This setting in culture started on the 30<sup>th</sup> of April.

*Musical diffusions.*

The music has been diffused in one of the vats, by mean of an aquatic speaker Altec UW-30, while the other vat served as a control.

The proteins transcribed in musical sequences were the following:

- TAPE I (45 min)
  - NIF H of *Anabaena v.* (five times)
  - Allophycocyanin of *Anabaena v.* (three times)
  - Plastocyanin of *Anabeana v.* (three times)
  - Nitrate reductase of *Chorella s.* (three times)
  - PS1 photosystem protein of *Anabaena v.* (three times) (\*)
  - Ferredoxin of *Anabaena v.* (five times)
  - Protein 35 K of *Anabaena v.* (eight times) (\*).
- TAPE II (15 min)
  - Allophycocyanin of *Anabaena v.* (two times)
  - Plastocyanin of *Anabaena v.* (two times)
  - PS1 photosystem protein of *Anabaena v.* (three times) (\*)
  - Ferredoxin of *Anabaena v.* (four times)
  - Protein 35 K of *Anabaena v.* (eight times).
- TAPE III (15 min)
  - Ferredoxin of *Anabeana v.* (two times)
  - NIF H of *Anabeana v.* (three times)

NIF A of *Anabeana* v. (three times) (\*)

NIF D of *Anabeana* v. (three times) (\*)

Nitrate reductase of *Chlorella* s. (three times)

Protein 35 K of *Anabeana* v. (two times) (\*).

The transcriptions had been realized by J. Sternheimer on a sampler Casio SK1 apart from those labelled (\*) which were made by P. Ferrandiz on a « One Key Play » software written by Sylvie Guillou and Fabrice Ocelli (INSERM St-Anne, Paris). The rate of the transpositions is tuned so as to make their length correspond to the photoperiods of the micro-organisms.

Tape I was played twice a day, from the 30<sup>th</sup> of April to the 5<sup>th</sup> of May. Then from the 7<sup>th</sup> to the 10<sup>th</sup> of May TAPE II was played in the morning while TAPE III was played in the evening.

During this period the viability of the micro-organisms was regularly controlled: Samples were drawn from the cultures and then checked under a microscope.

- Results (Annex 3)

- Evolution of the coloration of cultures (Figure 1)

One poured in the vats the solutions looked opaque (after tossing).

This was due to the manure mentionned above, the dilution rate of the original stock but also to the spread of a fibrous contaminant which was not characterized.

From the 2<sup>nd</sup> day of listening the musical vat presented a greater proportion of suspending matter than the control one. However this trend reversed itself by the 4<sup>th</sup> day. We therefore assumed that the musical exposures had been too long and we decided to abort the diffusion of Tape I.

Instead Tapes II and III have been used. We then observed on May 8<sup>th</sup> that the tint of the cultures in the musical vat displayed a green blue coloration more pronounced than those in the control vat (Figure 2).

This trend kept increasing up to the end of the experiment.

- Oxygen release.

Ten days after the end of the period of diffusion the musical cultures became characterized by a proliferation of bubbles at the surface (Figure 3).

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Since these bubbles had the property to revive the flame of a lighted match which was put close by, we concluded they contained oxygen. On May 24<sup>th</sup> there were about 70 surface bubbles and on the 28<sup>th</sup> they were 130 (Figure 4). We point out that the maximum number of visible bubbles observed in the control vat is 8. Hence there is more than a factor 16 between the two cultures with respect to oxygen release. In fact the medium of the musical culture was saturated with oxygen at the end of the observation time. Clearly this is correlated to an increase of the photosynthetic activity in the musical vat. It indicates that while the oxygen was released some carbonated composites have been fixed (Figure 5, taken six months later). Thus this particular application of the epigenetic regulation process led to an interesting depollutive system. This should beget further interests.

Other experiences showing the utility of the instant invention are herewith attached (Annexes 4-8).

As regards a garden experience: See Annex 9.

Figure 1 of this Annex 9 also attached is a comparative test:

On the left side (control): non treated tomatoes

On the right side: tomatoes having received during 16 days 3 minutes per day, the music of protein of anti-drought protein TAS14. Both control tomatoes and the treated tomatoes having 1 1½ litres of water per plant per day.

3. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

4. Further declarant saith not.

Joël Sternheimer JSN

Joël STERNHEIMER

1999/5/19

Date

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